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Running on Ran: Nuclear Transport and the Mitotic Spindle

Minireview

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Far from being passive participants in the process of mitosis, chromosomes have an active and essential role in the assembly of spindles required for their accurate division into the two daughter cells. One way in which they exert this influence is through the selective stabilization of microtubules (MTs) in their immediate vicinity, thereby contributing to the nucleation and growth of spindle MTs, as well as to their directed elongation from nucleating structures such as centrosomes (reviewed in Andersen, 1999). The mechanistic details of how chromosomes promote MT stability remained an intriguing mystery until the surprising discovery that the Ran GTPase is an essential component of this process (reviewed by Kahana and Cleveland, 1999).

Ran is a very abundant and highly conserved GTPase (reviewed in Sazer and Dasso, 2000). Previous data had implicated Ran in many aspects of nuclear function, including nuclear transport, cell cycle control, and postmitotic nuclear assembly. The finding that Ran regulates spindle assembly immediately posed the question as to how a single protein could be involved in such a diverse array of cellular activities and whether the effectors for each of these roles would be similar or distinct. Recent reports published in January issues of *Cell* (Gruss et al., 2001; Nachury et al., 2001) and *Science* (Wiese et al., 2001) shed light on this question and allow the proposal of a much more unified view of Ran's biochemistry throughout the cell cycle.

The Biochemistry of Ran and Its Role in Nuclear Transport

The core biochemistry of Ran is similar to that of many Ras-related GTPases (reviewed in Gorlich and Kutay, 1999; Sazer and Dasso, 2000). Ran's intrinsic rates of nucleotide exchange and hydrolysis are slow. In vivo, these reactions require a nucleotide exchange factor (RCC1) and a GTPase activating protein (RanGAP1) to occur at physiological rates. A protein called RanBP1 binds to Ran-GTP with high affinity, and acts as an essential accessory factor to increase RanGAP1-mediated nucleotide hydrolysis. During interphase, RCC1 is a chromatin-associated nuclear protein, while the bulk of RanBP1 and RanGAP1 are cytosolic. The asymmetric distribution of nucleotide exchange and hydrolysis enzymes across the nuclear envelope predicts that Ran-GTP should be largely nuclear and Ran-GDP should be largely cytosolic. This distribution plays a key role in determining the directionality of nuclear transport.

The requirement for Ran in nuclear transport has been extensively studied (reviewed in Gorlich and Kutay, 1999). A family of related Ran-GTP binding proteins act as receptors for both nuclear import and export. Import receptors associate with their cargo in the cytosol, where Ran-GTP concentrations are low, and dissociate from their cargo after entering the nucleus and binding Ran-GTP. Conversely, export receptors bind their cargo in complexes containing Ran-GTP within the nucleus and dissociate from their cargo after transit to the cytosol and GTP hydrolysis. In particular, nuclear import is accomplished for proteins bearing classical nuclear localization signals (NLS) by a heterodimeric receptor comprised of a Ran-GTP binding protein, importin β, and an adaptor protein, importin α (Figure 1A). Importin α recognizes NLS-bearing proteins. Importin β binds to the importin α-NLS complex and facilitates its movement through the nuclear pore. Importin β's use of adaptor subunits in this manner is unusual, since transport receptors generally bind to their cargo directly. Importin β is responsible for the translocation of other cargoes through direct association. Importin β dissociates from importin α-NLS after binding Ran-GTP in the nucleus, and returns to the cytosol in association with Ran-GTP. Importin α dissociates from NLS within the nucleus, and is then exported to the cytosol in association with an importin β–related export receptor, CAS, and Ran-GTP.

The Relationship between Ran and Mitotic Spindle Assembly

MTs are noncovalent polymers, composed of α- and β-tubulin dimeric subunits (reviewed in Desai and Mitchison, 1997). MTs alternate abruptly and stochastically between phases of polymerization and depolymerization. This behavior has been termed "dynamic instability." Dynamic instability is characterized by four parameters: polymerization rate, depolymerization rate, frequency of transition from growth to shrinkage (catastrophe frequency), and frequency of transition from shrinkage to growth (rescue frequency). MT dynamics are highly regulated during the cell cycle. The rate of catastrophe increases 5- to 10-fold upon entry into mitosis, causing mitotic MTs to be much shorter and more dynamic than interphase MTs (Desai and Mitchison, 1997). The MT nucleation capacity of centrosomes is also markedly enhanced as cells enter mitosis (Kuriyama and Borisy, 1981). Mitotic chromosomes locally decrease catastrophe frequency and increase rescue frequency, promoting the elongation of spindle MTs (Dogterom et al., 1996). A number of experiments have suggested that chromosome-induced MT stabilization requires the product of a chromatin-associated enzyme, which becomes distributed by diffusion into a gradient that is inversely proportional to distance from the chromosome (Andersen, 1999).

Ran-GTP promotes MT polymerization within M phase–arrested *Xenopus* egg extracts (Kahana and Cleveland, 1999). The addition of high levels of Ran-GTP, constitutively GTP-bound Ran mutants (RanQ69L, RanL43E), or exogenous RCC1 promotes massive spontaneous polymerization of tubulin, even in the absence of sperm chromatin or centrioles. Ran-GTP-induced MTs become arranged through the action of dynein and other motors into asters that contain typical centrosome-associated

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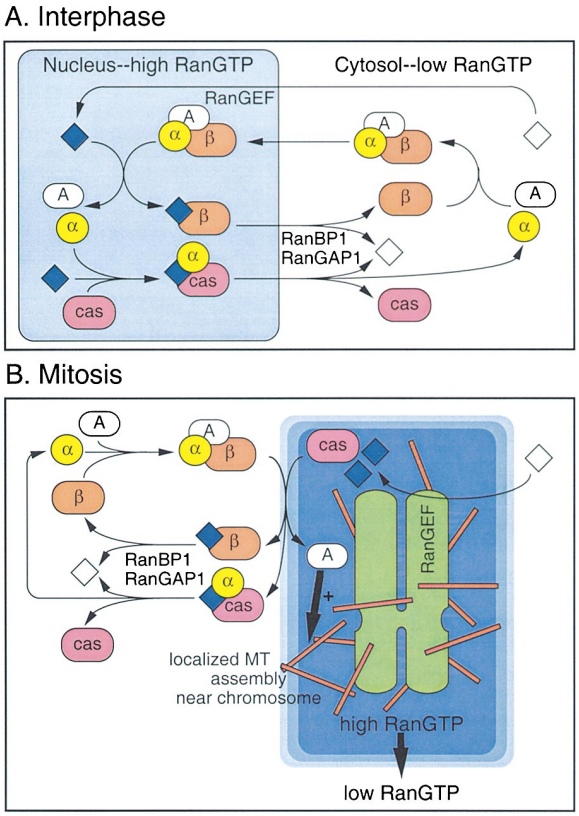

Figure 1. Schematic Model for Ran in Transport and Mitosis

(A) During interphase, Ran promotes NLS-mediated protein import of APA components through the association and dissociation of transport complexes. A complex containing aster promoting activities (A), importin α (α) and importin β (β) forms in the cytosol and translocates across the nuclear pore. In the nucleus, Ran-GTP (filled diamonds) binds to importin β and dissociates the transport complex. Ran-GTP and importin β shuttle back to the cytosol, where Ran-GTP is hydrolyzed by cytosolic RanGAP1 and RanBP1 to Ran-GDP (open diamonds). CAS (cas) and Ran-GTP bind to importin α in the nucleus, and this complex shuttles back to the cytosol, where it is also dissociated by the action of RanGAP1 and RanBP1. The polarized distribution of Ran-GTP across the nuclear envelope is maintained by the compartmentalization of RCC1 (indicated as RanGEF), RanGAP1, and RanBP1. (B) The localization of RCC1 on chromatin promotes MT assembly in mitosis. During mitosis, importin α and importin β sequester APA in an inactive form in regions where Ran-GTP is low. Increased levels of Ran-GTP near the chromosome (green) promote local disassembly of APA components from complexes containing importin α and importin β. In high Ran-GTP regions, importin β associates with Ran-GTP, maintaining it in an inactive state until RanGAP1 and RanBP1 catalyze Ran-GTP hydrolysis. After dissociation from importin complexes, APA becomes active in promoting MT (pink bars) assembly.

proteins (e.g., γ-tubulin, NuMA, and XGRIP109). Over time, these Ran-GTP-induced asters rearrange into bipolar structures resembling spindles. Since a significant fraction of RCC1 remains bound to chromatin in mitosis, it has been hypothesized that Ran-GTP could be the diffusionally distributed MT stabilization factor near mitotic chromosomes.

Ran probably has multiple targets in mitotic spindles. Carazo-Salas et al. (2001) analyzed behavior of centrosomal MTs in M phase egg extracts treated with RanQ69L. They concluded that RanQ69L increases rescue frequency while decreasing catastrophe frequency. Using similar analysis, Wilde et al. (2001) concluded that rescue frequency is increased in extracts containing another GTP-bound Ran mutant, RanL43E. Carazo-Salas et al. (2001) also tested whether RanQ69L can alter centrosomal MT nucleation capacity by incubating demembranated sperm in egg extracts containing nocodazole. This treatment allows the sperm centrosomes to become competent for nucleation but blocks MT elongation. The sperm heads were subsequently reisolated and incubated with purified tubulin. Sperm heads showed a significantly greater capacity to nucleate MTs if RanQ69L was present during the extract incubation, suggesting that Ran-GTP positively regulates MT nucleation.

Finally, there are indications that Ran-GTP alters the activity of MT motor proteins that organize MTs within spindles. For instance, asters assembled after treatment of extracts with agents that simply stabilize MTs, like DMSO or taxol, do not similarly rearrange with time into spindle-like structures (Wilde and Zheng, 1999). Moreover, Wilde et al. (2001) have assayed the mobility of rhodamine-labeled MT seeds in RanL43E-treated egg extracts. These measurements showed an increase in plus end–directed seed movement that was dependent upon the Eg5 motor protein. As will be discussed further below, recent evidence also provides a biochemical link between Ran-GTP and two motor–associated proteins, TPX2 and NuMA (Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001).

Rans Share Similar Effectors in Nuclear Transport and Spindle Assembly

In order to understand Ran's role in spindle assembly, Gruss et al. (2001) reasoned that transport receptors would be good candidates to act as effectors in this process. If this were the case, cargoes that compete for receptor binding should prevent association of aster promoting activities (APA) with the key receptor. Consistent with this idea, model substrates with NLSs promoted aster assembly in egg extracts, while cargo from other receptors did not. From this observation and other data, they concluded that importin α plays a critical inhibitory role in regulating APA, and that this inhibition is released when importin α-NLS complexes are dissociated by Ran-GTP and CAS. Removal of importin α binding proteins from egg extracts by affinity chromatography supported this conclusion and allowed the development of an assay for APA. Purification of an APA from HeLa cell nuclear extract gave a single candidate protein, TPX2 (Gruss et al., 2001). TPX2 is an MT-associated protein (MAP) (Wittmann et al., 2000) that is required for both Ran-GTP-induced aster assembly and assembly of spindles around DNA beads (Gruss et al., 2001). TPX2 is known to bind Xklp2, an MT motor protein, and to target it to spindles (Wittmann et al., 2000). However, it seems likely that TPX2 has other essential activities that are regulated by Ran, since removal of Xklp2 alone does not abolish spindle formation (Wittmann et al., 2000).

Following somewhat similar logic, Nachury et al. (2001) removed Ran-GTP binding proteins from egg extracts through their affinity for RanQ69L, and found that asters now formed in a spontaneous and Ran-GTP-independent manner. From this observation, they concluded that a Ran-GTP binding protein(s) is inhibitory to aster

Table 1. Mechanisms of Ran Function and Targets of Ran

Process	Role of Ran	Effector	Target	References
Nuclear transport	Cargo loading and unloading from transport receptor complexes	Importin α/β and Importin β-related Ran-GTP binding proteins	Multiple nuclear proteins and shuttling proteins	See Gorlich and Kutay, 1999 for review
Spindle assembly	Positive regulation of tubulin polymerization, Regulation of MT motor proteins	Importin α/β	TPX2 NuMA (Eg5-indirect target?)	Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001; Wilde et al., 2001; Carazo-Salas et al., 2001
Postmitotic nuclear assembly	Fusion of vesicles to form nuclear envelope	Unknown (Note: both nucleotide exchange and hydrolysis on Ran are required)	Unknown	Zhang and Clarke, 2000; Hetzer et al., 2000
Cell cycle control	Prevention of premature activation of cdc2/cyclin B when unreplicated DNA is present	Unknown	Unknown	See Sazer and Dasso, 2000 for review

formation and that its removal or inactivation by Ran-GTP allows MT polymerization. By adding back different transport receptors, they discovered that importin β inhibits aster formation in extracts depleted of Ran-GTP binding proteins, while other transport receptors did not. Using extracts depleted of both RanQ69L binding proteins and importin β cargo, it was possible to assay APA. In this assay, a fragment of NuMA (NuMA Tail II) restored aster assembly. NuMA is a MAP that associates with cytoplasmic dynein and that is essential for spindle assembly (Merdes et al., 1996). Purified NuMA Tail II associates with importin α and β, and this complex is dissociated by Ran-GTP, consistent with the notion that NuMA is a Ran target in spindle assembly (Nachury et al., 2001).

Wiese et al. (2001) came to essentially similar conclusions from the opposite direction. They were aware of previous reports that exogenous NuMA Tail II could promote the assembly of asters in egg extracts that were structurally similar to those assembled after addition of RanL43E (Merdes et al., 1996; Wilde and Zheng, 1999). They examined which egg extract proteins associate with NuMA Tail II, and discovered that importin β binds in a manner that could be released by Ran-GTP. Further experiments showed that importin β could antagonize formation of both RanL34E- and NuMA Tail II-induced asters. From these data, they concluded that NuMA's capacity to positively regulate MT assembly is inhibited by formation of importin β-NuMA complexes, and that Ran-GTP releases this inhibition by dissociating these complexes. Notably, while Gruss et al. (2001) showed that exogenous importin α could block aster assembly, Wiese et al. (2001) commented that they did not see inhibition with importin α addition. It is difficult to reconcile these two reports without better information about the experimental conditions used by Wiese et al. (2001).

On the basis of their findings, all three groups proposed similar models for the function of Ran in spindle assembly (Figure 1B). Namely, that importin α and β bind and inhibit APA required for the formation of stable MTs. In the vicinity of chromosomes, a high local concentration of Ran-GTP relieves this suppression through dissociation of importin α–importin β–APA complexes. As a result, APA are free to promote the assembly of MTs. In regions distal from the chromosomes, RanBP1 and RanGAP1 promote nucleotide hydrolysis within complexes of importin β-Ran-GTP, and importin α-CAS-Ran-GTP, resulting in free importin α and β that can again sequester APA. The binding of importin α and β to APA would cause APA to be sequestered to the nucleus during interphase. This may be a mechanism to assure that they are not inappropriately active within interphase cytosol. Notably, quantitative sequestration into complexes with importin α and β was not demonstrated for either TPX2 or NuMA in untreated egg extracts. In order to achieve such quantitative association, these proteins would need to bind importin α and β with unusual avidity, given the high concentration of other NLS-bearing proteins that could compete for receptor binding. This is a point that is relatively central to the model and should be experimentally addressed in the near future.

A number of other mitotic MT regulators, including XKCM1, XCTK2, XMAP310, and MKLP-1/CHO1, are localized to the nucleus throughout interphase. It will be of interest to determine how many of these proteins or other APA are controlled by the mechanism proposed above. Gruss et al. (2001) found that TPX2 was fully sufficient to complement aster formation in extracts depleted of importin α binding proteins. They concluded that TPX2 is the single essential importin α binding protein that acts as a Ran-dependent APA, in apparent contradiction to the findings of Nachury et al. (2001) and Wiese et al. (2001). It is difficult to resolve this point at present. It may be that excess recombinant TPX2 used to complement depleted extracts has sufficient aster promotion activity to obscure any requirement for other proteins, such as NuMA, which might be functionally redundant or which might be only partially depleted by importin α affinity columns. The notion of redundancy would be supported by the very similar phenotypes obtained after the addition of TPX2 or NuMA tail II to egg extracts (Merdes et al., 1996; Wittmann et al., 2000). In this regard, it should also be noted that NuMA-depleted egg extracts can assemble spindle MTs, but are deficient in focusing them into a bipolar spindle structure

(Merdes et al., 1996), suggesting that NuMA cannot be the only protein required for chromatin-induced MT stability. In addition, Nachury et al. (2001) have raised the possibility that some APA may interact with importin β in an importin α–independent manner, and these activities will also be important to investigate.

Could This Be a General Mechanism for Other Roles of Ran?

One attractive feature of the model discussed above is that it offers a unified picture of Ran's biochemistry in spindle assembly and nuclear transport. In both cases, spatial cues are given through changes in the concentration of Ran-GTP. For transport, high Ran-GTP promotes import receptor unloading and export receptor loading in the nucleus. For spindle assembly, high Ran-GTP promotes the specific activation of APA in the vicinity of chromatin through dissociation of complexes containing the same receptor molecules in their mitotic guise.

It will be interesting to determine how well this model is conserved evolutionarily, particularly in organisms that do not undergo an open mitosis. The original experiments demonstrating a role for Ran in spindle assembly were conducted in *Xenopus* egg extracts. However, recent experiments from Guarguaglini et al. (2000) show that Ran has a role in spindle assembly in mammalian tissue culture cells and indicate that Ran's role is probably conserved among all metazoans. This conclusion is further supported by spindle disruption in cells injected with mutant importin β proteins that lack the capacity to bind Ran (Nachury et al., 2001). It is clear that Ran's role in nuclear transport is well conserved between budding yeast and metazoans (Gorlich and Kutay, 1999). Recent evidence also suggests that Ran has a role in spindle MT integrity in fission yeast that can be distinguished from its role in nuclear transport (Fleig et al., 2000). However, there is no evidence of dissipation of the Ran-GTP gradient at mitosis in either budding or fission yeast, and RanGAP1 and RanBP1 remain cytosolic throughout the yeast cell cycle (reviewed in Sazer and Dasso, 2000). Spindle assembly occurs within the yeast nucleus, where the concentration of GTP-Ran should be uniformly high throughout the cell cycle, so it is difficult to imagine how a gradient of GTP-Ran might be established. Although Ran may promote spindle assembly in both yeast and metazoan, it therefore appears likely that there will be important differences in how divergent organisms utilize Ran in this process.

The theme established for Ran's biochemistry in nuclear transport and spindle assembly could certainly be expanded with a number of further variations. Ran has well-established roles in postmitotic nuclear envelope assembly (Hetzer et al., 2000; Zhang and Clarke, 2000) and in regulating the onset of mitosis (reviewed in Sazer and Dasso, 2000) (Table 1). However, the effectors for these processes are uncharacterized at present. Additionally, Nachury et al. (2001) noted that chromosomal morphology is disrupted in PtK1 cells injected with mutant forms of importin β that are deficient in Ran binding, leading them to speculate that chromatin condensation may also be directly or indirectly regulated by Ran. Linking several aspects of chromosomal metabolism through a single molecule could have foreseeable advantages for coordinating their regulation through the cell cycle. In principle, importin β or other transport receptors could act in any of these processes, and this paradigm will clearly be extensively tested in the near future.

However, there is one key aspect of this model has been poorly demonstrated experimentally—namely, the structure of the Ran-GTP gradient. During interphase, a wealth of circumstantial evidence supports the notion that Ran-GTP is primarily distributed to the nucleus (Gorlich and Kutay, 1999; Sazer and Dasso, 2000), but direct evidence of this distribution has been lacking, largely due to technical considerations. The existence of a Ran-GTP gradient in mitosis is entirely inferred from the localization of RCC1, and experimental limitations will make the mitotic Ran-GTP distribution even more difficult to document. It will be important not only to verify the existence and nature of the Ran gradient, but also to show how it is remodeled during the cell cycle as the nuclear envelope breaks down and reforms and as chromosomes condense and decondense. Given the central role now proposed for Ran-GTP gradients, it is reasonable to assume that Ran-GTP distribution will be closely regulated. Such regulation may help to account for earlier data implicating phosphorylation in chromatin-induced MT stabilization (Andersen, 1999). In the end, understanding Ran-GTP distribution may indeed provide the keys to the engine that is driving many machines.

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